USE OF OZONE IN STORAGE AND PACKING FACILITIES

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Ozone is a highly reactive form of oxygen where three molecules are bonded together. Generated electrically on-site where needed, it has potent antimicrobial activity and other characteristics. Interest in ozone applications for agriculture and food processing has increased in recent years (EPRI Expert Panel 1997). In 2001, ozone was declared a GRAS (generally recognized as safe) substance by the FDA after a Food Additive Petition containing safety and efficacy data was submitted to them. Later, the USDA approved its use on meats and on certified organic foods. In the mid-1990s, ozone was approved for food processing in Japan, France, and Australia. Ozone has a long history as a water disinfectant, and it is in common use for this purpose in many parts of the world. Many aspects of ozone use have been reviewed: (1) water disinfection applications (Nickols and Varas 1992; White 1999; Rice 1999); (2) food safety and sanitation (Graham et al. 1997; Kim, Yousef and Dave 1999), (3) chemistry (Razumovski and Zaikov, 1984), (4) responses of horticultural products to ozone (Forney 2003); and (5) the practical aspects of the design and operation of ozonators (Rice and Netzer 1984). The purpose for this article is to describe experiments where ozone applications in packinghouses have been evaluated.

The units that express concentration of ozone in air and water are typically parts per million (ppm). In water, ppm is a unit of weight/volume (µg/mL), while in air, ppm is a unit of volume/volume (µL/L). A liter of water with a concentration of 1 ppm ozone has many ozone molecules, while a liter of air with a concentration of 1 ppm ozone contains relatively few. When calculated, equal volumes of 1 ppm of ozone in water contain 500,000 times as many molecules as in 1 ppm ozone in air.

Ozone in Air

Conceivable benefits of adding ozone to air in packinghouses and storage rooms include control of postharvest diseases on fruit, retarding the production of spores from decaying fruit, sanitation of surfaces, and ethylene removal. Both benefit (Jin et al. 1989; Harding 1968; Liew and Prange, 1994; Palou et al. 2002; Palou et al. 2003; Pinilla et al. 1996; Sarig et al. 1996; Skog and Chu 2001) and lack of benefit (Hopkins and Loucks 1949; Spalding 1966; Spalding 1968) of ozone in air use in fruit and vegetable storage rooms have been reported. Forney (2003) recently reviewed this subject.

Schomer and McColloch (1948) summarized the risks and benefits of air ozonation in apple storage. Ozone did not control decay of apples, and it did not reduce infection of inoculated wounds, although it did retard the rate of enlargement of the infected areas. Spores of *Penicillium expansum* unprotected by fruit tissue or other organic matter were killed by continuous exposure to ozone. Colonies established on package surfaces, however, were very resistant and were not killed by continuous exposure for 5 months to an atmosphere containing 3.25 ppm ozone. Scald was not controlled by ozone, but its development was reduced. Fruit were injured by a daily exposure to 3.25 ppm ozone. The exposure period that caused injury and the degree of injury varied with the variety. No fruit were injured by daily exposure for five months to 1.95 ppm. The flavor of all varieties tested except Golden Delicious was impaired by 3.25 ppm of ozone, but no impairment was caused by 1.95 ppm on any variety tested. The cuticle of some varieties became sticky and varnish-like in the presence of ozone. No differences in the physiological properties between treated and untreated fruit were detected.

This work is quite complete and agrees with that of other workers. An issue in many older reports is that they did not use dry air in their ozone generators, so they may have produced ozone and other oxidants such as hydrogen peroxide, nitrous oxide, and nitric acid (Peyrous 1990) that may be responsible for some of the effects they observed. The effect of low ozone concentrations (0.3 to 1.0 ppm) on other fruit has been reported. Observations include a slight but significant inhibition by ozone of the rate of decay of oranges and lemons by *Penicillium digitatum* and *P. italicum* (Palou et al. 2001), of strawberries by *Botrytis cinerea* (Nadas at al. 2003), and peaches by *Monilinia fructicola* (Palou et al. 2001). Ozone at these low rates retarded the production of spores from infected fruit or cultures (Harding 1968; Palou et al. 2001; Palou et al. 2003; Nadas et al. 2003). Spore production is retarded when the gas is present, and resumes when the fruit are removed from the atmosphere. *B. cinerea* spores prepared from colonies grown in ozonated air were as infectious to strawberries as those from air (Nadas et al. 2003). Inhibition of spore production can be valuable because the population of spores within the packinghouse that can cause additional cycles of infection and decay are not produced. In some citrus packinghouses today, a high proportion of the green mold spores present are resistant to the fungicides available for use. Decay lesions caused by resistant fungi produce copious spores, in spite of the presence of ample fungicide residues, and exacerbate the build-up of resistant spores and decay losses. In these cases, ozone could retard the production of these spores when no other method is available. Sporulation control with ozone has been repeatedly demonstrated when the citrus fruit are in cold storage at 50 °F or less (Palou et al. 2001).

A fundamental issue is penetration by ozone into fruit containers. Ozone penetration into most conventional citrus packages is poor and adequate penetration occurs only packages with large vents or open tops (Harding 1968; Palou et al. 2003). Palou et al. (2003) showed in a room containing 0.7 ppm ozone, penetration into returnable plastic containers with large vents was good. The ozone concentration inside them was 0.6 ppm, and good sporulation control was evident. However, penetration into oranges with plastic bags or fiberboard cartons was 0.1 ppm or less and sporulation was not controlled.

Sanitation of equipment and fruit surfaces with ozone gas has been reported, but doses of ozone that kill postharvest pathogenic fungi in a few hours or days are very high and to use them requires very ozone-tolerant products, corrosion-resistant facilities that contain the gas, and presumably other measures to scrub ozone from vented air and other safety measures. We exposed spores on glass slides, then rinsed off the spores on to agar media to determine their germinability. We used glass slides because we found spores exposed to ozone on agar media typically die at much lower doses than those on hard surfaces (Li and Wang 2003). To kill spores of the pathogens that cause green mold, blue mold, and sour rot (*Penicillium digitatum*, *P. italicum*, and *Geotrichum citri-auranatii*, respectively) in humid air (about 95% RH) at 5 °C within one hour, we found about 200 ppm ozone was required. If the air was dry (35% RH), a dose 5 to 10 times higher was required. The feasibility of using ozone gas to rapidly kill fungi on fresh fruit is limited because in a survey of more than 60 fresh products we found many were injured by 200 ppm applied for one hour. Onions, citrus fruit, russet potatoes, cantaloupes, waxed apples, and kiwi fruit were unharmed when examined one week after treatment, while stone fruit, mushrooms, bananas, leafy vegetables of many kinds, snow peas, mangos, broccoli, brussels sprouts, and un-waxed apples and pears were severely harmed. A device built by PureOx Co. (Sparks, NV) can deliver 10,000 ppm ozone or more into a leak-proof chamber, and it uses a partial vacuum to facilitate gas penetration. Intended primarily for durable products such as spices, research to determine its ability to control insects and microorganisms are in progress.

The rapid reaction of ethylene and ozone in air is a well-documented phenomenon (Dickson et al. 1992), and for those commodities that benefit by ethylene removal, ozone may be of use. Recent work by Skog and Chu (2001) showed ozone could effectively prevent ethylene accumulation in apple and pear storage rooms at 0.4 ppm. Mushrooms, apples, pears, broccoli, and cucumbers tolerated this low concentration without harm. Although published studies to document the benefit of ethylene removal for citrus fruit are few (Wild et al. 1976), reducing ethylene to very low levels during citrus fruit storage results in less decay and prolongs storage life. Some devices function by scrubbing ethylene and spores from storage room air that passes through a corona device, so the ozone concentration in the storage room air is not necessarily elevated. Other oxidation products than ozone, particularly oxides of nitrogen, can be emitted if the air that passes into a corona discharge ozone generator is not dry, and these other products may have some role (Jin et al. 1989).

Ozone in Water

Ozone in water is often described as an alternative to hypochlorite as a disinfectant or sanitizer, although they differ in many aspects (Table 1). Significant advantages of ozone in water are that it decomposes quickly to oxygen, leaving no residue of itself and few disinfection by-products, and it has more potency against bacteria, cysts of protozoa, viruses, and fungal spores than hypochlorite (White 1999). Ozone can oxidize many organic compounds, particularly those with phenolic rings or unsaturated bonds in their structure (Razumovski and Zaikov 1984) and can therefore have a role in reducing pesticide residues in process water (Nickols and Varas 1992) and mycotoxins in durable commodities (McKenzie et al. 1997). These attributes make ozone a good choice when processed water is recycled. However, ozone in water is a dissolved gas and its solubility is relatively low compared to many other sanitizers, typically only about 30 ppm at 68 °F (20 °C). The presence of ozone-reactive compounds in the water, from soil or fruit constituents, can cause a significant ozone demand and cause the concentration to plummet quickly. Pre-conditioning of the water (to reduce particulates, organic compounds, turbidity, etc.) before ozonation, is needed in systems where water is recycled or the source water is of poor quality. In practice, even with high quality water, it is difficult to exceed 10 ppm, and many systems produce 5 ppm or less. However, spores are killed with relatively small doses. A contact time of two minutes in 1.5 ppm ozone killed 95 to 100% of the spores of eight fungi we tested, and none survived 3 minutes of contact (Figure 1). Ozone in water above 1 ppm can liberate ozone to the air that exceeds levels safe to workers, particularly if the water is warm and it passes through high-pressure, small-droplet size nozzles. A well-designed shroud over the application area with an ozone destruct device or a fan that discharges this air outside manages ozone off-gassing.

Ozonation to sanitize packingline process water. Ozone has been employed successfully in flume water in apple and pear packinghouses (Tukey 1993; Strasser 1998). In general, the water in tanks where fresh fruit are dumped or floated before cleaning, sorting, and packing operations is an important site for the accumulation of pathogens that infect fruit as they pass through the tanks so they rot later in storage, shipping, or marketing. Therefore, disinfection of this water is important, and usually is accomplished with sodium or calcium hypochlorite (chlorine). Ozone can replace chlorine for sanitation but several technical challenges must be addressed. Spores of all postharvest pathogens die quickly in ozonated water, but fruit, pear floatants, soil, and other debris in the water can reduce the ozone concentration rapidly to very low and ineffective levels. The ozone generator must work harder to produce enough ozone to compensate for that lost, and because only relatively clean water can be ozonated effectively, soiled water must pass through conditioning steps (such as flocculation and filtration) before it can be ozonated again. Chlorine is also reduced under these conditions, but is much more resistant to soil and lost chlorine can be readily replaced from a barrel of concentrated hypochlorite solution. Ozone can minimize chemical and microbial contamination of process water that contacts the fruit during postharvest handling. Ozone is more reactive than chlorine and breaks down ringed structures common to many pesticides. Imazalil, thiabendazole, and sodium ortho-phenyl phenate rapidly disappeared in ozonated water in tests we did; more than 95% of all three fungicides were destroyed within 30 minutes. Ong and coworkers (1996) and Hwang and coworkers (2001) showed ozonated water dips and removed from 50 to 100% of azinphos-methyl (guthion), captan, formetanate hydrochloride (carzol), and mancozeb in solution and on fresh and processed apples.

Sanitation of the surface of fruit can be achieved with ozonated water. The effectiveness of sanitation with ozonated water sprayed over brushes is typically modest and only slightly better than sterile-water washing alone. Reductions in the number of viable *Penicillium digitatum* or *Geotrichum citri-aurantii* spores or natural microbe populations were 1 to 2 $\log_{10}$ units (90 to 99%) with ozonated water applied at 1 to 5 ppm, while chlorine applied similarly at 200 ppm (a common rate in citrus applications) reduced populations 2 to 3 $\log_{10}$ units (Smilanick et al. 2002A, 2002B). Achen and Yousef (2001) reduced Escherichia coli 0157:H7 populations on apple surfaces by 2.6 to 3.7 $\log_{10}$ units by 3 minutes treatment in bubbling ozonated water, although reductions in the stem and calyx regions were less than 1 $\log_{10}$ unit.

Ozone in water to control pathogens inoculated into wounds on fruit. Many postharvest pathogens use wounds on the fruit surface that are usually inflicted at harvest, to initiate infections that are visible some time later. These infections are typically controlled by fungicides applied soon after harvest by drenchers or on fruit packing lines. Ozonated water has not been effective in controlling infections from wounds inoculated before ozone treatment. An example of this type of infection is green mold of citrus, caused by Penicillium digitatum. In our tests with citrus fruit, control of this pathogen inoculated into wounds on fruit failed even after prolonged treatment with very high ozone concentrations in water, although the spores are killed within one or two minutes in ozonated water (Smilanick et al. 2002B; Figure 1). Pathogens are even more protected from ozone than microbes that reside on the product surface, presumably because of poor ozone penetration into the wounds, or the leakage of ozone-reactive substances or antioxidants that reduced ozone dosage inside the wounds. The inability to control infections on inoculated citrus fruit with ozone treatment in our tests agrees with the results of Spotts and Cervantes (1992) in their work with ozone in water treatment of pears. Ozone does not differ from other sanitizers in its inability to stop wound pathogens. Hypochlorite and chlorine dioxide at practical concentrations (200 µg/mL or less) did not control infections within inoculated wounds on citrus (Eckert and Eaks 1989; Smilanick et al. 2002A) or pear (Spotts and Peters 1980) fruit.

Safety

Ozone is toxic and workers must be protected from it. The federal exposure limit in workplaces for ozone gas, a time-weighted average during an eight-hour workday, is 0.1 ppm. The concentration that is "immediately dangerous to life and health" (IDHL) is 5 ppm. This is the maximum concentration for which there are approved respirators; higher rates than this are dangerous and require self-contained breathing equipment. To be in compliance with state and federal safety codes, the capability to determine ozone concentrations in air on-site is usually required.

Table 1. Comparison of various aspects of hypochlorite and ozone use in water.

Attribute	Hypochlorite	Ozone
Microbial potency	Kills plant pathogens and microbial saprophytes effectively. Some human-pathogenic, spore-forming protozoa resistant. Maximum allowable rates under regulatory control.	Kills plant pathogens and microbial saprophytes effectively, including spore-forming protozoa. Maximum rate limited by ozone solubility, difficult to exceed about 10 µg/mL.
Cost	Chemical cost low. Repeated delivery required, sometimes pH and concentration controller systems needed, minor maintenance and energy costs, chlorine storage issues. Need water of at least moderate quality.	Variable: no chemical cost, but high initial capital cost for generator, usually needs filtration system if water re-used Generators are complex, modest maintenance and energy costs. Must have high quality, clean water with low oxidation/reduction potential.
Influence of pH	Efficacy diminishes as pH increases, above pH 8, pH adjustment may be needed. Chlorine gas released at very low pH (4 or less.)	Potency not influenced very much by pH, but ozone decomposition increases rapidly above pH 8.
Disinfection by-products	Some regulatory concern, tri-halo compounds, particularly chloroform, of some human safety concern.	Less regulatory concern, small increase in aldehydes, ketones, alcohols, and carboxylic acids created from organics, bromate can form from bromine.
Worker safety issues	Chloramines can form and produce an irritating vapor. Chlorine gas systems require on-site safety measures OSHA (TWA) limit for chlorine gas: 1 µg/mL.	Off-gas ozone from solutions an irritant and must be managed. MnO_2 ozone destruction efficient and long-lived. OSHA (TWA) limit for ozone gas: 0.1 ppm (µL/L).
Persistence in water	Persists hours in clean water, persistence reduced to minutes in dirty water.	Persists minutes, clean water, persistence reduced to seconds in dirty water.
Use rates	Limited by regulation to 25 to 600 µg/mL, depending on application.	Not limited by regulation, but Henry's law limits theoretical maximum ozone in water to about 30 ppm (µg/L at 20 °C (68 °F). Most ozone systems produce 5 µg/mL or less.
Use in warm water	Increases potency, some increase in vapors.	Not practical, rapidly accelerates ozone decomposition, increases off-gassing, decreases ozone solubility.
Influence on product quality	Little risk of injury at recommended rates of 200 µg/mL or less.	In brief water and low concentration gas applications, risk of injury to citrus appears low, but needs more evaluation.
Impact on water quality	Minor negative impact: water salt concentration increases somewhat, may interfere with fermentation used to reduce Biological Oxygen Demand, some pesticides inactivated, discharge water dechlorination may be required.	Mostly positive impact: does not increase salt in water, many pesticides decomposed, Biological/Chemical Oxygen Demand may be reduced, flocculation and biodegradability of many organic compounds enhanced, precipitates iron, removes color, odors.
Corrosiveness	High, particularly iron and mild steel damaged.	Higher, particularly rubber, some plastics, yellow metals, aluminum, iron, zinc, and mild steel corroded.

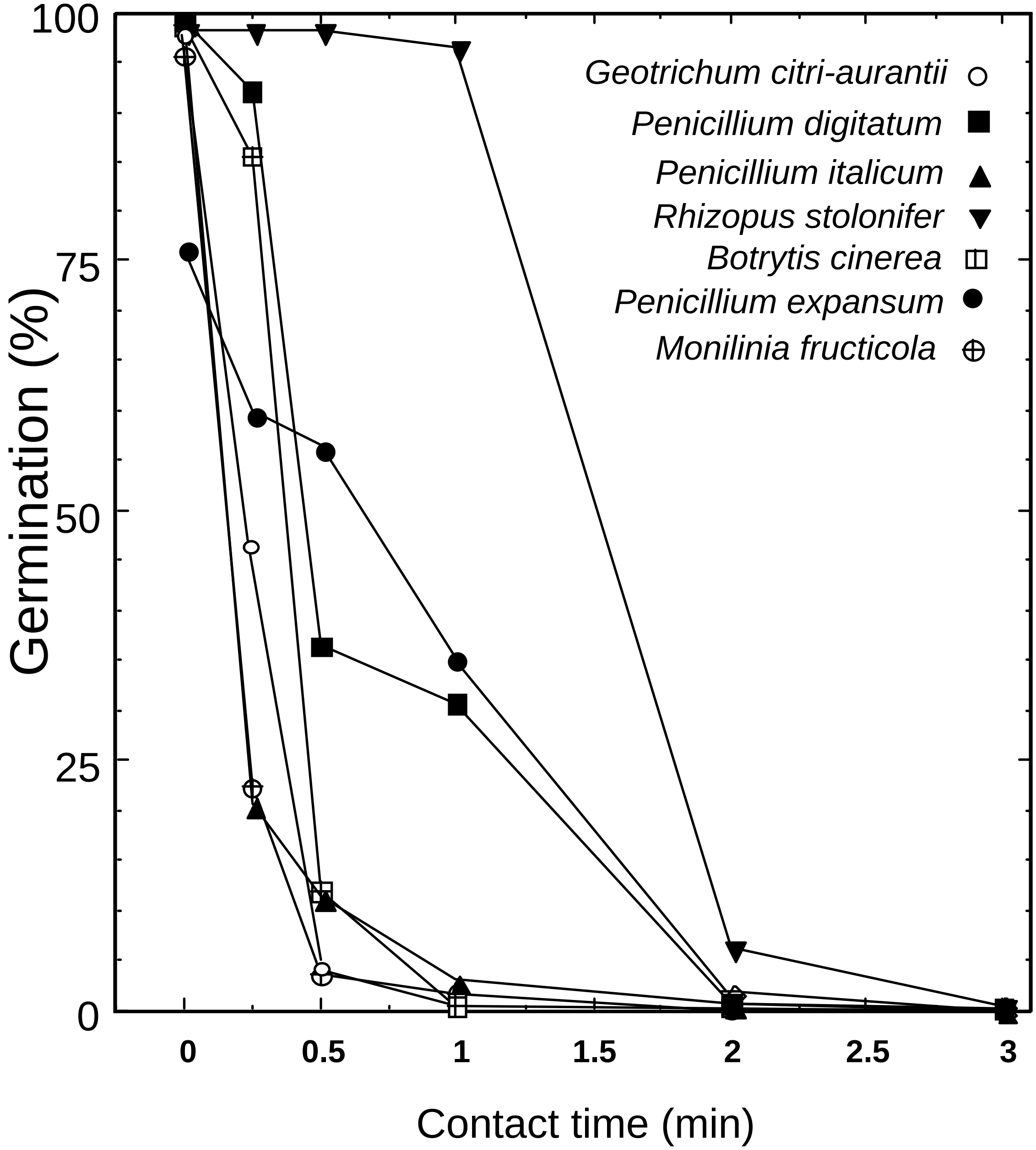

Figure 1. Germination of spores of various postharvest pathogenic fungi after exposure to 1.5 ppm (µg/mL) ozone in water at 16.5 °C (62 °F) and pH 6.4.

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